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Pathophysiology of chemical injury of the thyroid gland

C.C. Capen

Department of Veterinary Pathobiology, The Ohio State University, Columbus, OH (USA)

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SUMMARY

Many goitrogenic xenobiotics that increase the incidence of thyroid tumors in rodents exert a direct effect on the thyroid gland to disrupt one of several possible steps in the biosynthesis and secretion of thyroid hormones. This includes: (1) inhibition of the iodine trapping mechanism (thiocyanate or perchlorate); (2) blockage of organic binding of iodine and coupling of iodothyronines to form thyroxine (T_4) and triiodothyronine (T_3) (e.g. sulfonamides, thiourea, methimazole, and aminotriazole, amongst others); (3) inhibition of thyroid hormone secretion by an effect on proteolysis of active hormone from the colloid (lithium or an excess of iodide). Another large group of goitrogenic chemicals disrupts thyroid hormone economy by increasing the peripheral metabolism of thyroid hormones through an induction of hepatic microsomal enzymes. This group includes CNS-acting drugs (phenobarbital, benzodiazepines), calcium channel blockers (nicardipine, nifedipine), steroids (spironolactone), retinoids, chlorinated hydrocarbons (chlordane, DDT, TCDD), polyhalogenated biphenyls (PCB, PBB), and enzyme inducers. Thyroid hormone economy also can be disrupted by xenobiotics that inhibit the 5'-monodeiodinase which converts T_4 in peripheral sites (e.g. liver and kidney) to biologically active T_3. Inhibition of this enzyme by FD&C Red No. 3, amiodarone, and iopanoic acid lowers circulating T_3 levels which results in a compensatory increased secretion of thyroid stimulating hormone (TSH), follicular cell hypertrophy and hyperplasia, and an increased incidence of follicular cell tumors in 2-year or lifetime studies in rats. Physiologic perturbations alone, such as the feeding of an iodine-deficient diet, partial thyroidectomy, natural goitrogens in certain foods, and transplantation of TSH-secreting pituitary tumors in rodents also can disrupt thyroid hormone economy and, if sustained, increase the development of thyroid tumors in rats. A consistent finding with all of these goitrogens, be they either physiologic perturbations or xenobiotics, is the chronic hypersecretion of TSH which places the rodent thyroid gland at greater risk to develop tumors through a secondary mechanism of thyroid oncogenesis.

Correspondence to: C.C. Capen, Department of Veterinary Pathobiology, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210, USA.

INTRODUCTION: SPECIES DIFFERENCES IN THYROID HORMONE ECONOMY

Long-term perturbations of the pituitary–thyroid axis by various xenobiotics or physiologic alterations (e.g. iodine deficiency, partial thyroidectomy, and natural goitrogens in food) are more likely to predispose the laboratory rat to a higher incidence of proliferative lesions (e.g. hyperplasia and adenomas of follicular cells) in response to chronic thyroid stimulating hormone (TSH) stimulation than in the human thyroid [1,2]. This is particularly true in the male rat which has higher circulating levels of TSH than in females. The greater sensitivity of the rodent thyroid to derangement by drugs, chemicals and physiologic perturbations also is related to the shorter plasma half-life of thyroxine (T_4) than in man due to the considerable differences between species in the transport proteins for thyroid hormones [3].

The plasma T_4 half-life in rats is considerably shorter (12–24 h) than in man (5–9 days). In human beings and monkeys circulating T_4 is bound primarily to thyroxine-binding globulin (TBG) but this high affinity binding protein is not present in rodents, birds, amphibians or fish. The binding affinity of TBG for T_4 is approximately one thousand times higher than for prealbumin. The percent of unbound active T_4 is lower in species with high levels of TBG than in animals in which T_4 binding is limited to albumin and prealbumin. Therefore, a rat without a functional thyroid requires about 10 times more T_4 (20 μg/kg body weight) for full substitution than an adult human (2.2 μg/kg body weight). Triiodothyronine (T_3) is transported bound to TBG and albumin in human beings, monkey, and dog but only to albumin in mouse, rat, and chicken. In general T_3 is bound less avidly to transport proteins than T_4 resulting in a faster turnover and shorter plasma half-life in most species.

GOITROGENIC CHEMICALS AND THYROID TUMORS IN RODENTS

Numerous studies have reported that chronic treatment of rodents with goitrogenic compounds results in the development of follicular cell adenomas. Thiouracil and its derivatives have this effect in rats [4] and mice [5]. This phenomenon also has been observed in rats that consumed brassica seeds [6], erythrosine (FD&C Red No. 3) [1,7], sulfonamides [8], and many other compounds [9,10]. The pathogenetic mechanism of this phenomenon has been understood for some time and is widely accepted [11]. These goitrogenic agents either directly interfere with thyroid hormone production in the thyroid gland or increase thyroid hormone excretion into the bile. The ensuing decrease in circulating thyroid hormone levels results in a compensatory increased secretion of pituitary TSH. The TSH stimulation of the thyroid gland leads to proliferative changes of follicular cells that include

hypertrophy, hyperplasia, and ultimately, neoplasia in rodents.

Excessive secretion of TSH alone (i.e. in the absence of any chemical exposure) also has been reported to produce a high incidence of thyroid tumors in rodents. This has been observed in rats fed an iodine deficient diet [12] and in mice that received TSH-secreting pituitary tumor transplants [11]. The pathogenetic mechanism of thyroid follicular cell tumor development in rodents involves a sustained excessive stimulation of the thyroid gland by TSH.

HEPATIC MICROSOMAL INDUCTION AND THYROID TUMORS

Hepatic microsomal enzymes play an important role in thyroid hormone economy since glucuronidation is the rate limiting step in the biliary excretion of T_4 and sulfation by phenol sulfotransferase for the excretion of T_3. Long-term exposure of rats to a wide variety of different chemicals may induce these enzyme pathways and result in chronic stimulation of the thyroid by disrupting the hypothalamic–pituitary–thyroid axis. The resulting chronic stimulation of the thyroid by increased circulating levels of TSH often results in a greater risk of developing tumors derived from follicular cells in 2-year or lifetime studies with these compounds in rats.

Xenobiotics that induce liver microsomal enzymes and disrupt thyroid function in rats include central nervous system (CNS)-acting drugs (e.g. phenobarbital, benzodiazepines); calcium channel blockers (e.g. nicardipine, bepridil); steroids (spironolactone); retinoids; chlorinated hydrocarbons (e.g. chlordane, DDT, TCDD), polyhalogenated biphenyls (PCB, PBB), amongst others. Most of the hepatic microsomal enzyme inducers have no apparent intrinsic carcinogenic activity and produce little or no mutagenicity or DNA damage. Their promoting effect on thyroid tumors usually is greater in rats than mice with males more often developing a higher incidence of tumors than females. In certain strains of mice these compounds alter liver cell turnover and promote the development of hepatic tumors from spontaneously initiated hepatocytes.

Phenobarbital has been studied extensively as the prototype for hepatic microsomal inducers that increase a spectrum of cytochrome P450 isoenzymes [13]. McClain et al. [14] reported that the activity of UDP-glucuronyltransferase, the rate limiting enzyme in T_4 metabolism, is increased in purified hepatic microsomes of male rats when expressed as picomoles/min/mg microsomal protein (1.3-fold) or as total hepatic activity (3-fold). This resulted in a significantly higher cumulative (4 h) biliary excretion of ^{125}I-T_4 and bile flow than in controls. Phenobarbital-treated rats develop a characteristic pattern of changes in circulating thyroid hormone levels [13,14]. Plasma T_3 and T_4 are markedly decreased after one week and

remain decreased for 4 weeks. By 8 weeks T_3 levels return to near normal due to compensation by the hypothalamic–pituitary–thyroid axis. Serum TSH values are elevated significantly throughout the first month but often decline after a new steady state is attained. Thyroid weights increase significantly after 2–4 weeks of phenobarbital, reach a maximum increase of 40–50% by 8 weeks, and remain elevated throughout the period of treatment.

There is no convincing evidence that humans treated with drugs or exposed to chemicals that induce hepatic microsomal enzymes are at increased risk for the development of thyroid cancer. In a study of the effects of microsomal enzyme-inducing compounds on thyroid hormone metabolism in normal healthy adults, phenobarbital (100 mg daily for 14 days) did not affect the serum T_4, T_3, or TSH levels [15]. A decrease in serum T_4 levels was observed after treatment with either a combination of phenobarbital plus rifampicin or a combination of phenobarbital plus antipyrine; however, these treatments had no effect on serum T_3 or TSH levels [16]. Epidemiologic studies of patients treated with therapeutic doses of phenobarbital have reported no increase in risk for the development of thyroid neoplasia [17,18]. Highly sensitive assays for thyroid and pituitary hormones are readily available clinically to monitor circulating hormone levels in patients who are exposed to chemicals that could potentially disrupt homeostasis of the pituitary–thyroid axis.

CHEMICAL INHIBITION OF 5'-MONODEIODINASE AND THYROID TUMORS

Erythrosine (FD&C Red No. 3) is an example of a well-characterized xenobiotic that results in perturbations of thyroid function in rodents and in long-term studies is associated with an increased incidence of benign thyroid tumors. Red No. 3 is a widely used color additive in foods, cosmetics, and pharmaceuticals. A chronic toxicity/carcinogenicity study revealed that male Sprague-Dawley rats fed a 4% dietary concentration of Red No. 3 beginning *in utero* and extending over their lifetime (30 months) developed a 22% incidence of thyroid adenomas derived from follicular cells compared to 1.5% in control rats and a historical incidence of 1.8% for this strain [7]. There was not a significant increase in follicular cell adenomas in the lower dose groups of male rats or an increase in malignant thyroid follicular cell tumors. Female rats fed similar amounts of the color did not develop a significant increase in either benign or malignant thyroid tumors. Feeding of the color at the high dose (4%) level provided male rats with 2464 mg/kg of Red No. 3 daily; by comparison human consumption in the United States is estimated to be 0.023 mg/kg/day per person.

The results of mechanistic studies have suggested that a primary (direct) action of FD&C Red No. 3 on the thyroid is unlikely due to: (1) failure of the

color (^{14}C-labeled) to accumulate in the gland, (2) negative genotoxicity and mutagenicity assays, (3) lack of an oncogenic response in mice and gerbils, (4) a failure to result in thyroid tumor development at dietary concentrations of 1.0% or less in male and female rats [7], and (5) a lack of increased tumor development in other organs.

Subsequent investigations included a 60-day study in male Sprague-Dawley rats fed either 4% (high dose) or 0.25% (low dose) of FD&C Red No. 3 compared to controls in order to determine the effects of the color on thyroid hormone economy and morphometric changes in follicular cells. The experimental design of the study was to terminate groups of rats (n = 20/interval and dose) fed Red No. 3 and controls after 0, 3, 7, 10, 14, 21, 30, and 60 days. A consistent effect of Red No. 3 on thyroid hormone economy was the striking increase in serum reverse T_3. In the high-dose rats reverse T_3 was increased at all intervals compared to controls and at 10, 14, and 21 days in the low dose group. The mechanisms responsible for the increased serum reverse T_3 appear to be: first, substrate (T_4) accumulation due to 5′-deiodinase inhibition with subsequent conversion to reverse T_3 rather than active T_3; and second, reverse T_3 accumulation due to 5′-deiodinase inhibition resulting in an inability to degrade reverse T_3 further to diiodothyronine (T_2). Serum triiodothyronine (T_3) was decreased significantly at all intervals in rats of the high-dose group compared to interval controls. The mechanism responsible for the reduced serum T_3 following feeding of Red No. 3 was decreased monodeiodination of T_4 due to an inhibition of the 5′-deiodinase by the color.

Serum TSH was increased significantly at all intervals in rats of the high-dose (4%) group compared to controls. Rats fed 0.25% of Red No. 3 had increased serum TSH only at days 21, 30, and 60. The mechanism responsible for the increased serum TSH following ingestion of Red No. 3 was a compensatory response by the pituitary gland to the low circulating levels of T_3 that resulted from an inhibition of the 5′-deiodinase. Serum T_4 also was increased significantly at all intervals in rats fed 4% Red No. 3 compared to controls. The mechanism responsible for the increased serum T_4 was: first, accumulation due to an inability to monodeiodinate T_4 to T_3 in the liver and kidney from the inhibition of 5′-deiodinase by the color; and second, TSH stimulation of increased T_4 production by the thyroid gland.

^{125}I-labeled T_4 metabolism was significantly altered in liver homogenates prepared from rats fed 4% FD&C Red No. 3. Degradation of labeled T_4 was decreased to approximately 40% of values in control homogenates. This was associated with a 75% decrease in percent generation of ^{125}I and an approximately 80% decrease in percent generation of ^{125}I-labeled T_3 from radiolabeled T_4 substrate. These mechanistic investigations suggested that the color results in a perturbation of thyroid hormone economy in rodents by

inhibiting the 5'-deiodinase in the liver, resulting in long-term stimulation of follicular cells by TSH which over their lifetime predisposed to an increased incidence of thyroid tumors [1,7].

Morphometric evaluation was performed on thyroid glands from all rats at each interval during the 60 day study. Four levels of thyroid were evaluated with 25 measurements from each rat using a Zeiss interactive digital analysis system at a magnification of 450x. The direct measurements included diameter of thyroid follicles, area of follicular colloid, and height of follicular cells. Thyroid follicular diameter was decreased significantly in both low- and high-dose groups at 3, 7, 10, and 14 days compared to interval controls. The area of follicular colloid generally reflected the decrease in thyroid follicular diameter and was decreased significantly at days 3 and 10 in high-dose rats and days 7 and 10 in the low-dose group compared to interval controls. These reductions in thyroid follicular diameter and colloid area were consistent with morphologic changes expected from an increased serum TSH concentration.

Thyroid follicular height was increased significantly only after feeding FD&C Red No. 3 for 60 days in both the high- and low-dose groups compared to interval controls. The absence of morphometric evidence of follicular cell hypertrophy at the earlier intervals was consistent with the modest increase (+ 5.8%) in thyroid gland:body weight ratio after this relatively short exposure to the color. The lack of follicular cell hypertrophy at the earlier intervals of feeding Red No. 3 in rats with several fold elevations in serum TSH levels may be related, in part, to the thyroid iodine content. The thyroid responsiveness to TSH is known to vary inversely with iodine content [19,20]. Thyroid glands of rats fed FD&C Red No. 3 would be exposed to an increased iodine primarily from sodium iodide contamination of the color and, to a lesser extent, from metabolism of the compound and release of iodide.

SECONDARY MECHANISMS OF THYROID ONCOGENESIS

Understanding the mechanism of action of xenobiotics on the thyroid gland provides a more rational basis to extrapolate findings from long-term rodent studies to safety assessment of a particular compound for humans. Many chemicals and drugs disrupt one or more steps in the synthesis and secretion of thyroid hormones, resulting in subnormal levels of T_4 and T_3, associated with a compensatory increased secretion of pituitary TSH. When tested in highly sensitive species, such as rats and mice, these compounds result early in follicular cell hypertrophy/hyperplasia and increased thyroid weights, and in long-term studies an increased incidence of thyroid tumors by a secondary (indirect) mechanism.

In the secondary mechanism of thyroid oncogenesis in rodents the specific xenobiotic chemical or physiologic perturbation evokes another stimulus (e.g. chronic hypersecretion of TSH) that promotes the development of nodular proliferative lesions (initially hypertrophy, followed by hyperplasia, subsequently adenomas, infrequently carcinomas) derived from follicular cells. Thresholds for a no-effect on the thyroid gland can be established by determining the dose of xenobiotic that fails to elicit an elevation in the circulating level of TSH. Compounds acting by this indirect (secondary) mechanism with hormonal imbalance usually have little or no evidence for mutagenicity or for producing DNA damage.

When human patients have markedly altered changes in thyroid function and elevated TSH levels, as in areas with a high incidence of endemic goiter due to iodine deficiency, there is little if any increase in the incidence of thyroid cancer [2,21]. The relative resistance to the development of thyroid cancer in humans with elevated plasma TSH levels is in marked contrast to the response of the thyroid gland to chronic TSH stimulation in rats and mice. The human thyroid is much less sensitive to this pathogenetic phenomenon than rodents [2,14]. Human patients with congenital defects in thyroid hormone synthesis (dyshormonogenetic goiter) and markedly increased circulating TSH levels may have an increased incidence of thyroid carcinomas [22,23]. Likewise, thyrotoxic patients with Grave's disease where follicular cells are chronically stimulated by an immunoglobulin (Long Acting Thyroid Stimulator) also appear to be at greater risk to develop thyroid tumors [24,25]. In summary, the literature suggests that prolonged stimulation of the human thyroid by TSH will induce neoplasia only in exceptional circumstances, possibly acting together with some other metabolic or immunologic abnormality [2].

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